Analytical methods: Current and new methods: Chemical methods

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Chemical analysis of fish meal and fish oil

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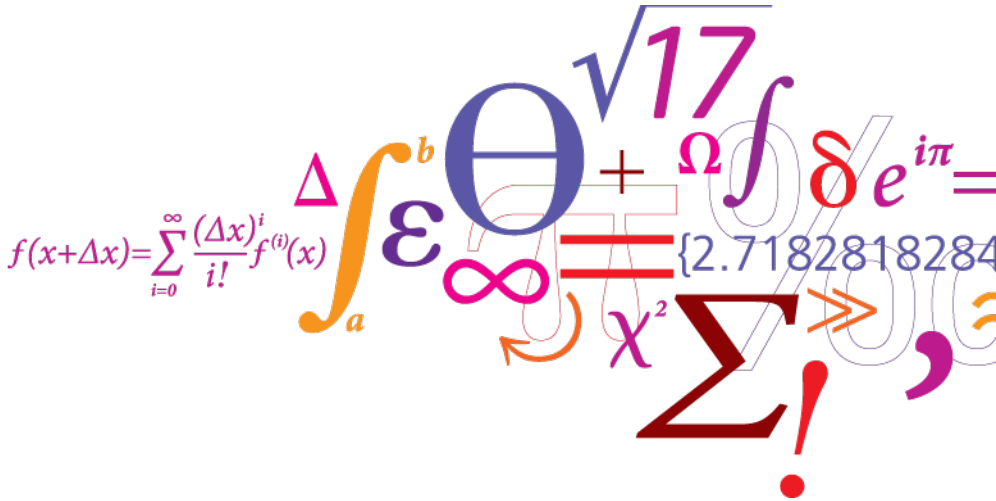


DTU Food
National Food Institute

Agenda

- TVN
- Biogenic amines
- Proteins (Kjeldahl vs Dumas)
- Free fatty acids
- PV (titration vs other spectrophotometric methods)
- AV
- TBARS
- Volatile oxidation products by headspace GC-MS

TVN

- The combined total amount of ammonia, dimethylamine and trimethylamine is called the total volatile base content of the fish (usually expressed as mg-N/l00 g minced fish) and is a commonly used estimate of spoilage

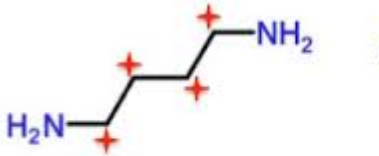
Conway method

- Make an aqueous acidic extract of the material
- Make the extract alcaline to release volatile bases
- Collect the bases in HCl and titrate with NaOH using Andersen indicator

- Can also be determined by steam distillation using Kjeldahl apparatus
- Can alse be determined by capillary electrophoresis

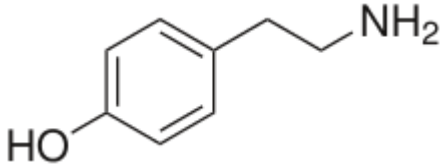
Biogenic amines

- Acidic extraction of biogenic amines (cadaverine, putrescine, tyramine and histamine)

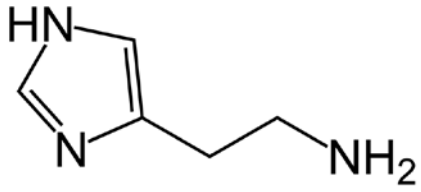


Putrescine
(1,4-Tetramethylenediamine)

Cadaverine
(1,5-Pentaminediamine)



Tyramine



Histamine

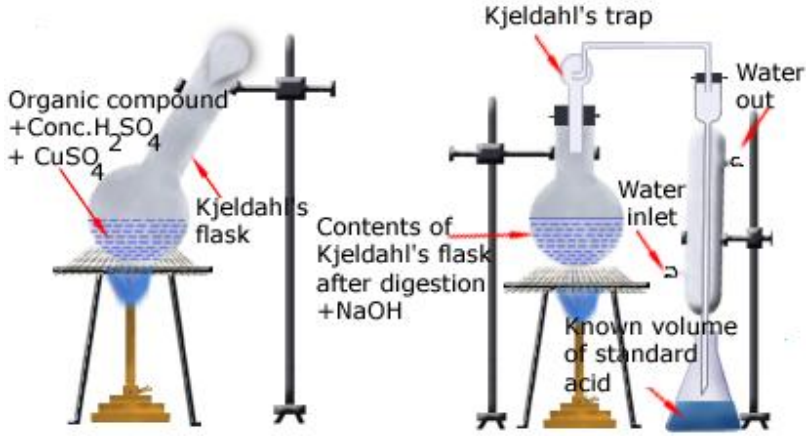
- HPLC analysis

- Analysis by Capillary Electrophoresis (CE) can also be performed

Protein determination in fish meal

- Kjeldahl *vs* Dumas

Kjeldahl



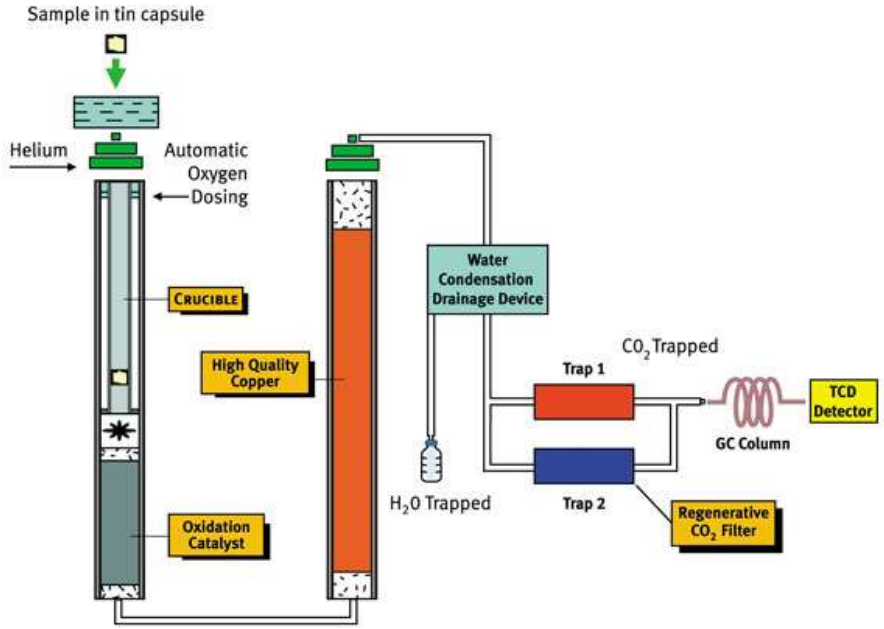
Advantages:

- widely used internationally and is still the standard method for comparison against all other methods
- universality, high precision and good reproducibility have made it the major method for the estimation of protein in foods

Disadvantages:

- It does not give a measure of the true protein, since all nitrogen in foods is not in the form of protein
- Different proteins need different correction factors because they have different amino acid sequences
- The use of concentrated sulfuric acid at high temperatures and heavy metal catalysts poses a considerable hazard
- The technique is time consuming to carry-out.

Dumas



Advantages:

- It is much faster than the Kjeldahl method (under 4 minutes per measurement, compared to 1-2 hours for Kjeldahl)
- It doesn't need toxic chemicals or catalysts
- Many samples can be measured automatically
- It is easy to use.

Disadvantages:

- High initial cost
- It does not give a measure of the true protein, since all nitrogen in foods is not in the form of protein.
- Different proteins need different correction factors because they have different amino acid sequences

Comparison of Dumas and Kjeldahl on different samples at DTU Food

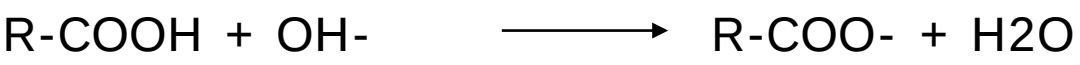
1: Microalgae
2: Fish
3: Oat beer
4: Barley beer
5: Malt beer

Protein content (g/100 g; N x conversion factor)

Samples	DTU (Kjeldahl)	Elementar (Dumas)	LECO 628 (Dumas)
Microalgae	44,1±1,1	51,92±0,08	48,21±0,09
Fish	21,43±0,07	22,48±0,53	22,09±0,03
Malt beer (B1)	0,25 – 0,28	----	0,31±0,003
Barley beer (B2)	0,15 – 0,31	0,24±0,008 *	----
Oat beer (B3)	0,51 – 0,60	----	0,18±0,003

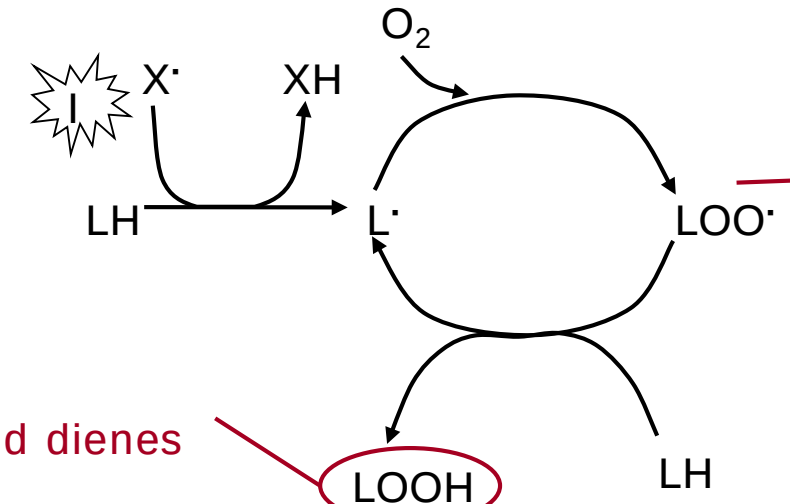
Free fatty acids

- Free fatty acids are titrated with NaOH with phenolphthalein as indicator



- pKa for fatty acids : 4-5

Measurement of lipid oxidation



TOTOX = 2 x PV + AV

GOED : TOTOX < 26

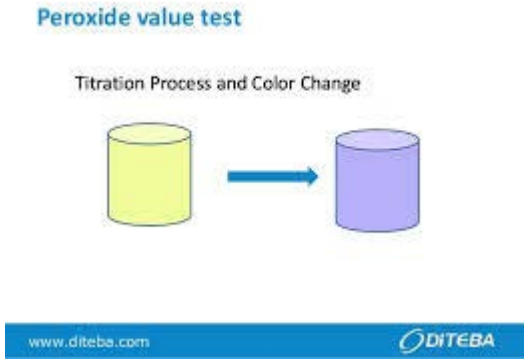
Peroxide value

For fish meal: Lipid extraction by chloroform and methanol to obtain lipid extract before PV analysis

PV (titration – colour change) (Standard method)

$$ROOH + 2I^- \rightarrow ROH + I_2$$

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$$



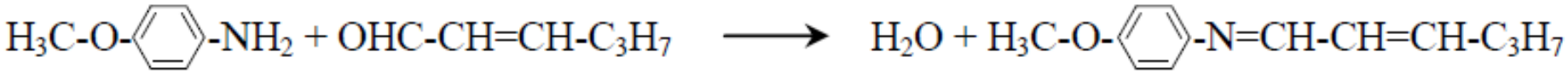
Different PV methods

	Modif IDF/ferro	Titration	Micro	FOX2
1	Ox of ferro-salts to ferri ions	Ox of iodide to free iodine	Ox of iodide to free iodine	Ox of ferro-salts to ferri ions
2	Production of red colour after addition of SNC^-	Titration with thiosulfate	Production of blue colour Iodine-starch complex	Production of blue colour complex (Ferri-Xyl-orange)
3	ROOH + Fe^{2+} →Fe^{3+} Fe^{3+} + $3SNC^-$ →complex	ROOH + $2I^-$ → ROH + I_2 I_2 + $2S_2O_3^{2-}$→$2I^-$ + $S_4O_6^{2-}$	ROOH+ $2I^-$ →ROH+I2 I_2+ starch → Incl.complex	ROOH+ Fe^{2+} →Fe^{3+} Fe^{3+}+ Xyl-or → complex
4	A 500nm		A 565nm	A 560nm
5	0.01-0.3 g / 0.1g	1 g	0.02-0.08 g	0.01-0.3 g
6				

1: Principle PV determination; 2: Detection; 3: Chemical reaction;
4: Absorption maximum coloured product; 5: Sample amount (oil); 6: Solvent volume, incl.complex: inclusion complex; Xyl-or: xylenol orange

AV – standard method oil industry

AV (spectrophotometric):



p-anisidine + aldehyde (fx: 2-hexenal) → coloured product

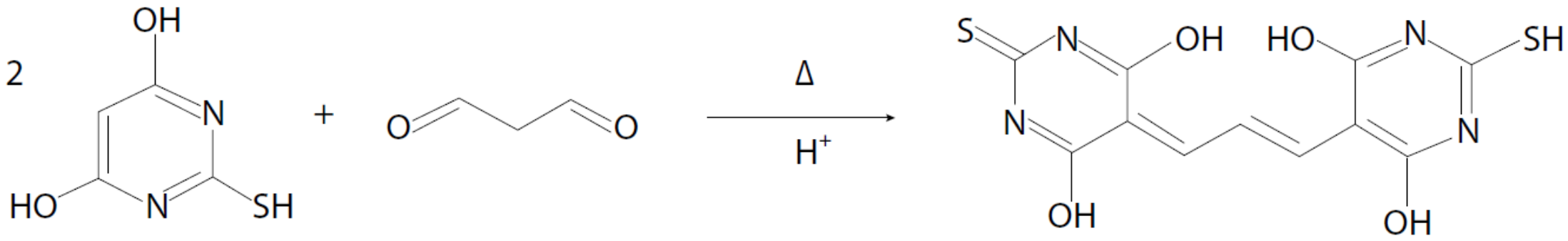
Colour intensity depends on the structure of the aldehydes!!

Thus, we do not really know what we measure

More sensitive and specific methods are therefore required, particularly for measurements of secondary oxidation products

TBARS

- Thiobarbituric acid reactive substances ”TBA(RS)”:



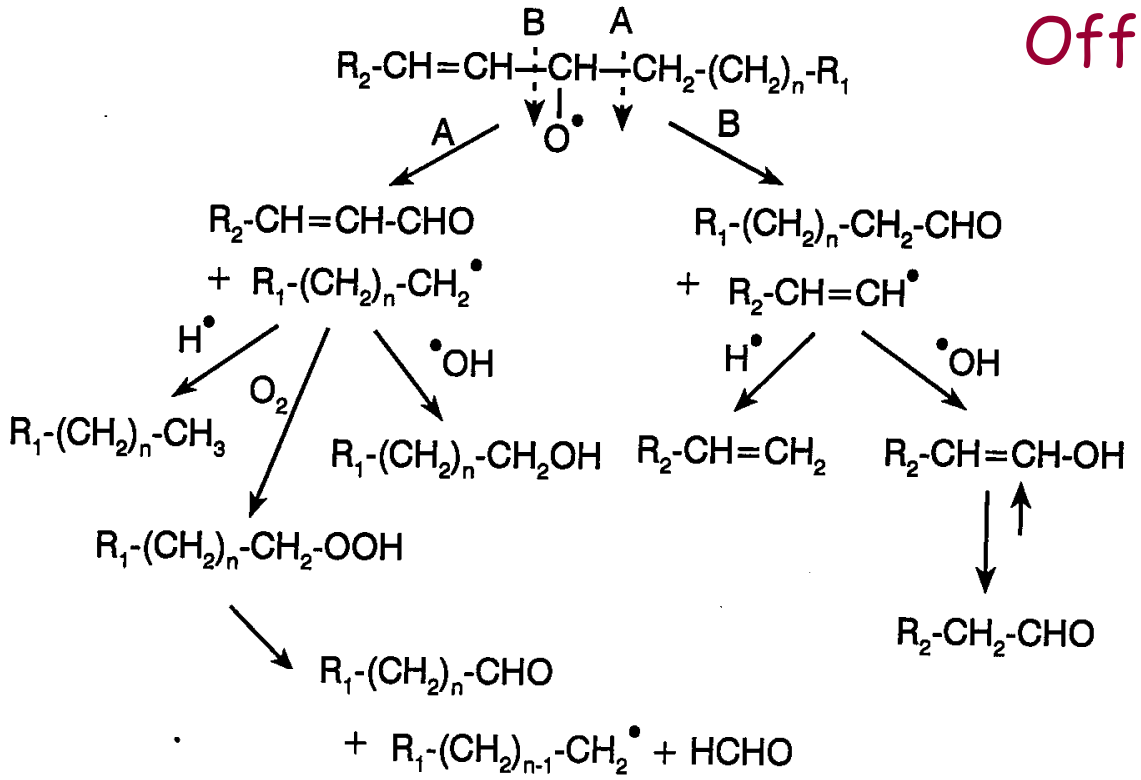
- TBA reacts with malondialdehyde, but pigment (535nm) is also formed with many other compounds (non-specific and interferences!)

Lipid hydroperoxide decomposition

Metal ions catalyzes this reaction

R_2 -↓- CH(O•) -↓- R_1 → Aldehydes + other volatiles

Off-flavors

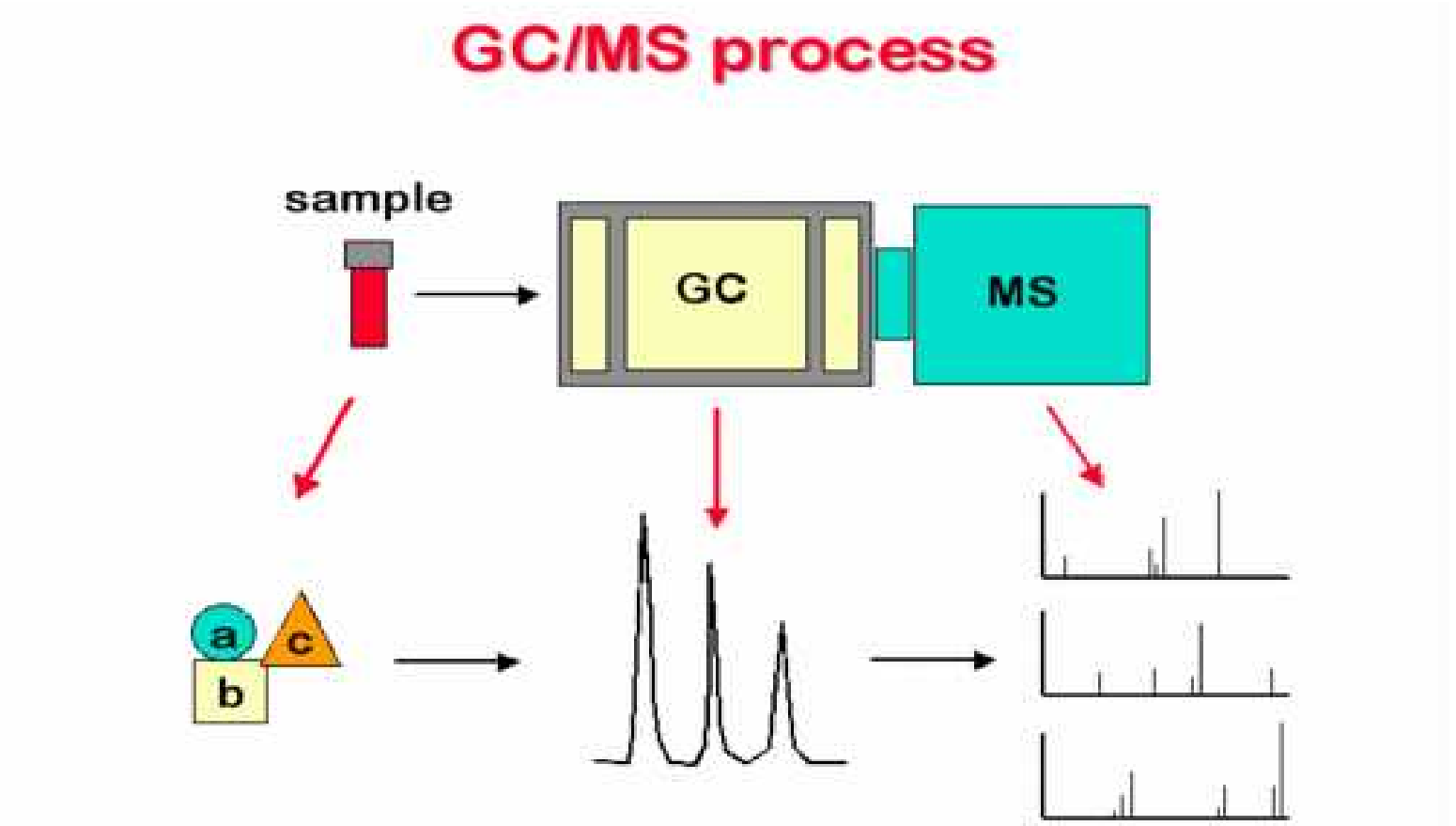


Aldehydes
Alkyl radicals
Olefin radicals

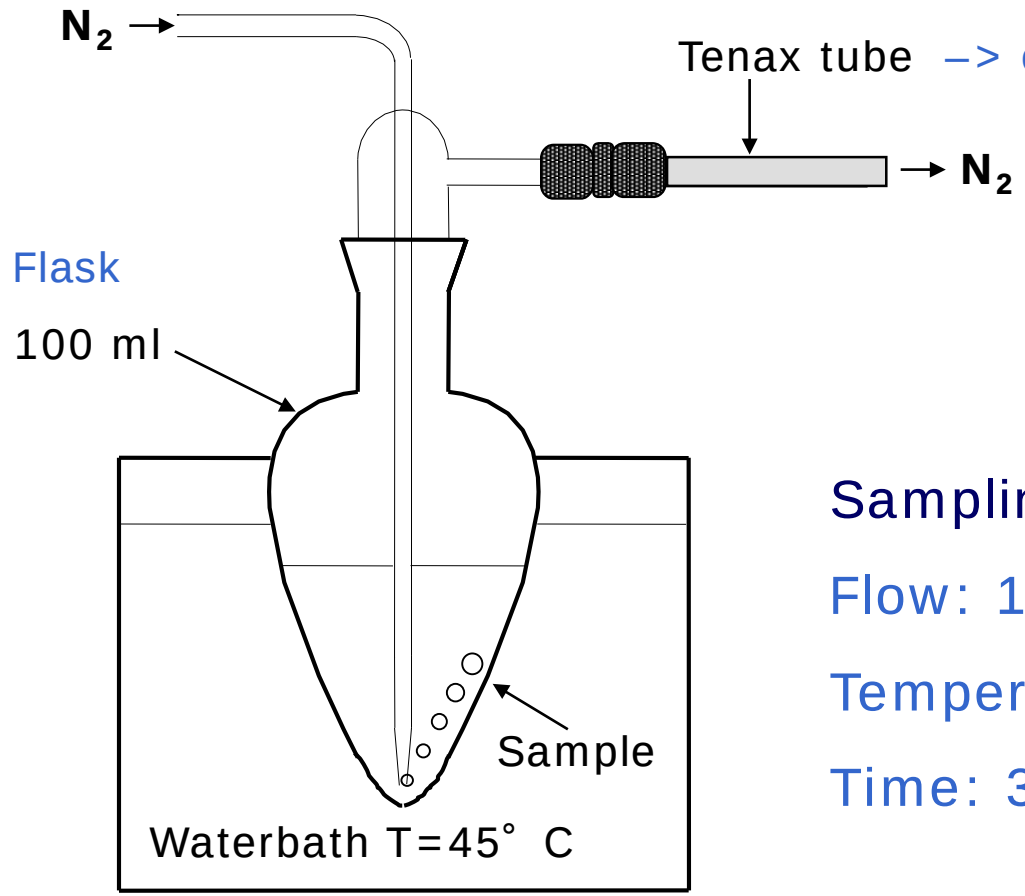
Frankel 1998

Gas chromatography - Mass spectrometry

- GC: separation of the different compounds -> chromatogram
- MS: analysis of the different compounds -> spectrum



Dynamic headspace sampling



Tenax tube inserted into

Automatic thermal desorber

Volatiles released and transferred to GC

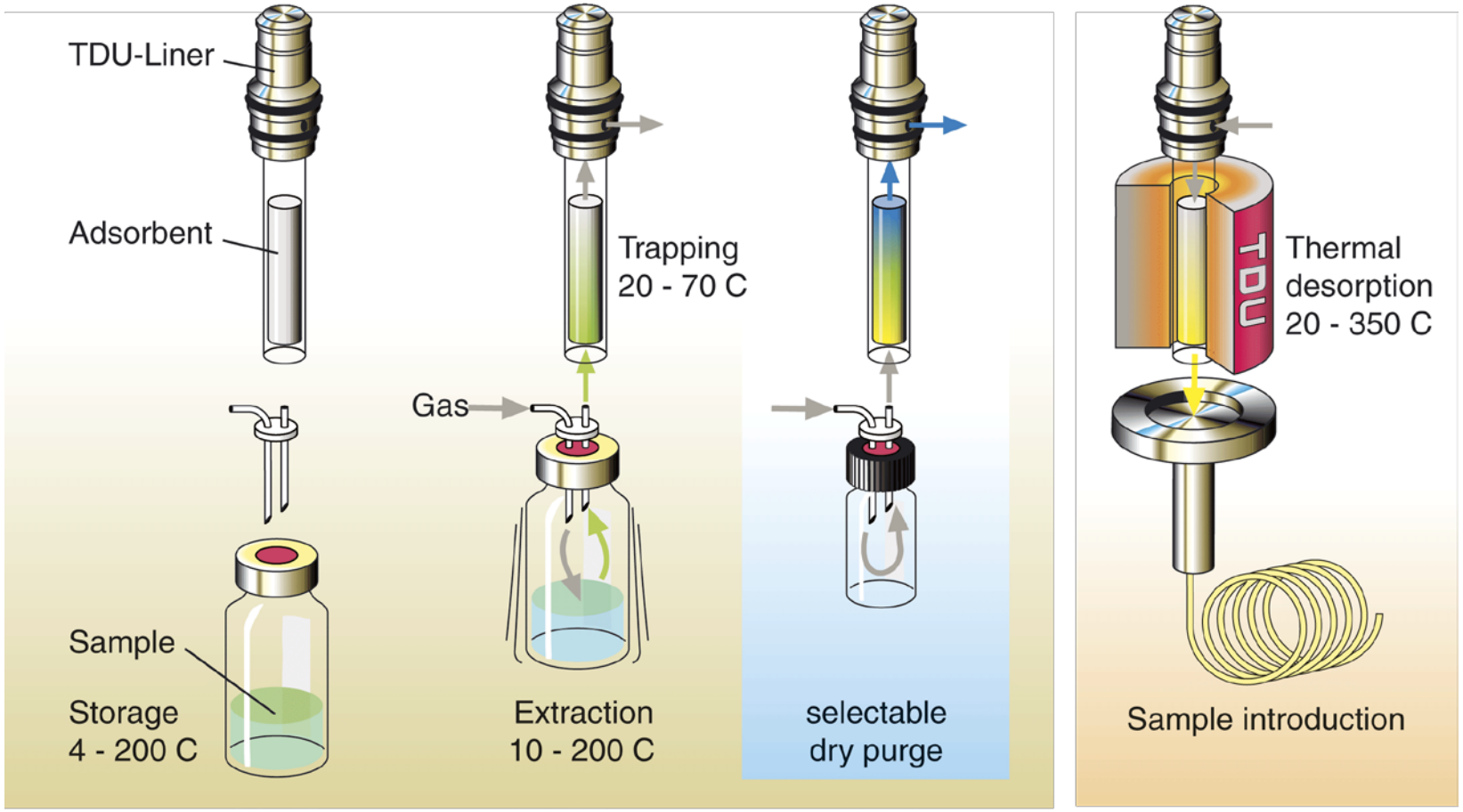
Sampling parameters:

Flow: 150 ml/min

Temperature: 60° C

Time: 30 min

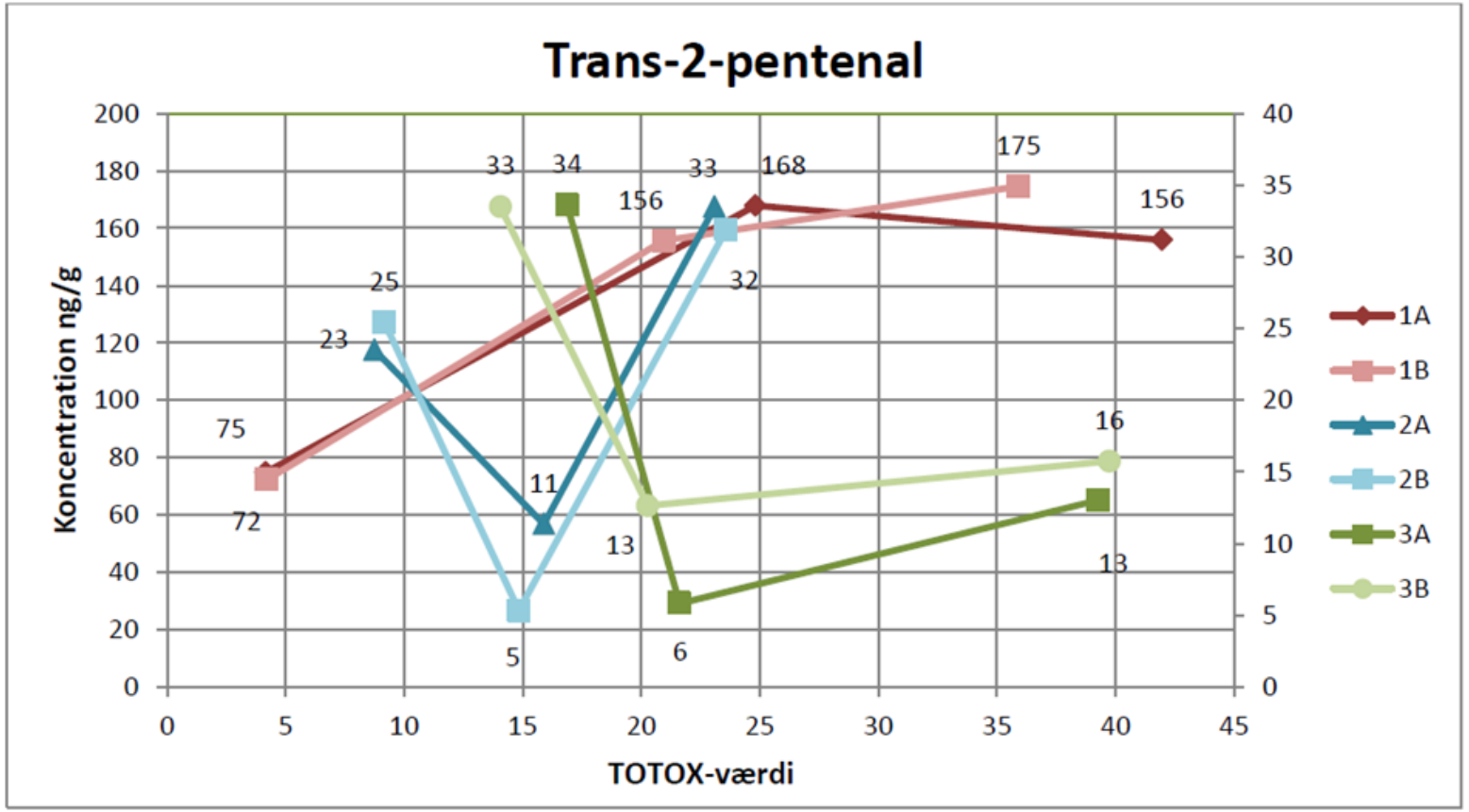
New automated TDU/DHS method



Courtesy: Gerstel GmbH & Co. KG

SPME and TDU sampling robot

Correlation between TOTOX and volatile oxidation products?



Challenges and research needs

- For fish meal
  - Standard method for protein determination using Dumas principle?

- For fish oil (for human consumption):
  - An alternative to the AV method is needed
  - For headspace GC-MS there is no standard method and labs are doing the analysis in many different ways

Thank you for your attention!

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